ORIGINAL ARTICLE

Analgesic activity of various extracts of *Punica granatum* (Linn) flowers

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The extracts of flowers of *Punica granatum* (Linn). (N.O. Family Punicaceae) were investigated for analgesic activity in mice using hot plate method. The flowers of *Punica granatum* (Linn) were collected from the local market of Mumbai, Maharashtra and were in a dried condition. The dried powdered flowers (500 gm) were extracted in a soxhlet apparatus by using different solvents. Mice weighing 15-25 gm were taken for the experiment. The reaction time of animals in all the groups was noted at 30, 60 and 120 min after drug administration. All data were analyzed with Student-t test. The various extract of the flowers of *Punica granatum* (Linn) showed significant analgesic activity at a dose of 50 mg/kg body weight. A maximum analgesic activity was found at 60 min, after drug administration, which was equivalent to the standard drug used as morphine sulphate.

Key words: Analgesic activity, mice, *Punica granatum* (Linn.)

INTRODUCTION

Punica granatum (Linn) is shrub or small tree belonging to the family Punicaceae. The tree is native from Iran to the Himalayas in northern India and has been cultivated since ancient times throughout the Mediterranean region of Asia, Africa and Europe.[1] The juice of the shrub yields citric acid and sodium citrate. It is useful in the treatment of dyspepsia and leprosy. The bark of the stem and root is useful in the treatment of worm infections. Dried flowers are used in the treatment of bronchitis. Decoction of the flower is used in cases of oral and throat inflammations. It contains certain chemical constituents like alkaloids: isopelletierine.[2] Apart from these uses leaves, seeds, roots and bark have displayed hypotensive, antispasmodic and anthelmintic activity in bioassay. It is also used as bactericide[3] and stimulant. It is given in cases of cardiac problems, inflammation and bleeding disorders. It is considered as the richest source of the female hormone estrone, which are precursors to phytoestrogens production in the body, along with tannins and ellagic acid, which are known for supporting the body functions.[4] The present investigation was aimed at evaluating the analgesic activity of the flowers of *Punica granatum* (Linn).

MATERIALS AND METHODS

Collection of Plant Material

The flowers of *Punica granatum* were collected from the local market areas of Mumbai, and they were sent for authentification and confirmed by Dr. M.P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen is kept in the Department of Pharmacognosy, SVKM's, NMIMS University, in School of Pharmacy and Technology Management.

Preparation of Extracts

The dried plant material flowers (500 gm) were subjected to hot continuous extraction with Chloroform, Methanol and Aqueous as solvents respectively. The various flower extracts were collected and concentrated to dry mass by using rotary vacuum evaporator. The dried mass was kept in a desicator and was used, as and when required for the experiment. The residue obtained from various extracts are Chloroform (100 gm), Methanol (320 gm) and Aqueous extract (240 gm) was used for this study.[5] The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in leaves.[6] It was observed that chloroform extract contained steroids whereas alcoholic and aqueous extracts contained steroidal saponins, flavonoids, tannins, phenolic substances and carbohydrates.

Animals

Adult Swiss albino mice of either sex, weighing between 15-25 gm were used for the study. They were grouped in to six, each consisting of six mice. All the animals were housed in animal house of the institution in polypropylene cages maintained under standard conditions (12 hourlight/12 hour dark cycle; 25 ± 2°C, 35-60% humidity and were handled in conformation with ethical guidelines. Prior permission from the

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Table 1: Analgesic activity of *Punica granatum* Linn

Group No.	Treatment	Basal reaction time in sec mean ± SEM	Reaction time in min. after injection of the extract (Mean ± SEM)		
			30	60	120
I	Control (Normal saline)	4.3 ± 0.076	4.01 ± 0.06	4.13 ± 0.07	4.23 ± 0.06
II	Solvent	4.23 ± 0.061	4.3 ± 0.05	4.15 ± 0.07**	4.11 ± 0.07
III	Morphine	4.63 ± 0.061	10 ± 0.73	11.83 ± 0.7	14 ± 0.73
IV	Chloroform extract	2.15 ± 0.04	5.2 ± 0.08*	14.66 ± 1.11**	13.66 ± 0.95**
V	Methanol extract	3.18 ± 0.06	6.78 ± 0.06**	16 ± 0.73**	14.66 ± 0.87**
VI	Aqueous extract	1.96 ± 0.06	6.5± 0.07**	15.83 ± 0.47**	13.33 ± 0.98**

Values expressed as mean + SEM, n = 6 in each group. *P < 0.05, **P < 0.01 compared to control

Maximum analgesic activity was obtained at 60 min., after the drug administration, which was found to be near to that of the standard drug. So it can be suggested that this might be due to the opioid receptors which are binding with the extracts. Further studies may be done for finding out the exact mechanism involved with the isolation of the components present in the extract by novel technologies

Institutional Animal Ethical Committee, was obtained as per the prescribed guidelines.

Evaluation of Analgesic Activity

The analgesic activity was assessed by Hot plate Technique. Swiss albino mice (18-25 g) were selected, weighed and divided into six groups of six animals each. All these animals were fasted 18h prior to commencement of experiment but water was provided *ad libitum.* Animals of group I received the normal saline 1 ml/kg. Group II received solvent. Group III received morphine sulphate 5 mg/kg by IP route. Group IV received chloroform extract with the solvent in a dose of 50 mg/kg by IP route. Group V received methanol extract with the solvent in a dose of 50 mg/kg by IP route. Group VI received aqueous extract in sterile water for injection in a dose of 50 mg/kg by IP route. Each mouse was picked on the hot plate which was maintained at constant temperature 55°C. The basal reaction time, the time taken for the jump response or paw licking which ever appears to be first was observed. The reaction time of animals in all groups was noted at 30, 60 and 120 min after the drug administration. The cut off time of 15 sec was taken as maximum analgesic response to avoid injury to the paws. The percentage increase in reaction time at each time interval was calculated.[7]

Statistical Analysis

The results were subjected to statistical analysis using ordinary ANOVA followed by Dunnett's multiple comparison test. All the data are presented as mean ± SEM [Table 1]. The P values < 0.001 were considered significant.

RESULTS AND DISCUSSION

Results of analgesic study showed that the various extracts of *Punica granatum* (Linn.) were significant at dose of 50 mg/kg. The result of the activity on mice by hot plate method are given in Table 1. The basal reaction time of the standard drug, chloroform, methanol and aqueous extracts of *Punica granatum* (Linn) treated animals was increased. Thus various extracts of *Punica granatum* (Linn) showed a statistically significant activity with respect to the reference treated drug. The mechanism by which analgesic effect occurs is not fully understood. Inhibition of prostaglandin may not be the main factor, but may be one of the several possibilities.

CONCLUSION

The analgesic activity of *Punica granatum* (Linn.) supports its use in the traditional medicine to reduce pain but further studies are needed to elucidate the exact mechanism by which *Punica granatum* (Linn) flower extract exerts the analgesic effect.

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